PCT/DE2003/003028

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Hec-008 PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DE2003/003028	International filing date (*day/month/year*) 12 September 2003 (12.09.2003)	Priority date (*day/month/year*) 12 September 2002 (12.09.2002)
International Patent Classification (IPC) or national classification and IPC C12N 15/11		
Applicant AVONTEC GMBH		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17 March 2004 (17.03.2004)	24 September 2004 (24.09.2004)
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/DE2003/003028

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-31, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:

pages 1-10, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the drawings:

pages 1/8 - 8/8, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the sequence listing part of the description:

pages 1-23, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

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III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-3 (in part), 4-10 (completly)

because:

☐ the said international application, or the said claims Nos. _____
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 1-3 (in part), 4-10 (completly).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
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IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☐ the parts relating to claims Nos. _____ .

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes III and IV.

Owing to lack of unity of invention, the ISA has searched only the first invention (claims 1-3 (all in part)). No further search fees were paid and consequently no further searches were carried out. The examination therefore relates exclusively to the searched subject matter (PCT Rule 66.1(e)).

This report refers to the following documents:

D1: Miyamoto Y. et al.: "Replication protein A1 reduces transcription of the endothelial nitric oxide synthase gene containing a -786T→C mutation associated with coronary spastic angina", HUMAN MOLECULAR GENETICS, Vol. 9, No. 18, 1 November 2000, pages 2629-2637

D2: WO 01 53537 A (Moskowitz David W.; DZGENES LLC (US)) 26 July 2001

D3: Nakayama M. et al.: "T-786-C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene associated with coronary spasm", CIRCULATION, Vol. 99, 1999, pages 2684-2870

D4: Cattararuzza M. et al.: "The -786C variant of the human endothelial nitric oxide synthase gene promoter is a risk factor of coronary heart disease", PFLUEGERS ARCHIV EUROPEAN JOURNAL OF PHYSIOLOGY, Vol. 443, No. Supplement 1, March 2002 (2002-03), page S255, 81st Annual

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes III and IV.

Joint Meeting of the Physiological Society, the Scandinavian Physiological Society and the [...]; Tübingen, Germany; March 15-19, 2002

D5: Dötsch J. et al.: "Increase of endothelial nitric oxide synthase and endothelin-1 mRNA expression in human placenta during gestation", EUROPEAN JOURNAL OF OBSTETRICS, GYNECOLOGY, AND REPRODUCTIVE BIOLOGY. IRELAND, August 2001, Vol. 97, No. 2, August 2001 (2001-08), pages 163-167.

1.1 The International Searching Authority has determined that the international application contains multiple (groups of) inventions that are not so linked as to form a single general inventive concept (PCT Rule 13.1), that is:

Invention 1 (claims 1-3 (in part)):
Decoy oligonucleotide with a nucleic acid sequence according to Seq. Id. No. 1 or 2. Said oligonucleotide as drug. Said oligonucleotide for the manufacture of a drug for preventing or treating one of the diseases specified in claim 3.

Inventions 2-17 (claims 1-3 (in part))
As Invention 1, but in each case relating to the next two in succession from Seq. Id. No. 3-34.

Invention 18 (claims 4-9 (in full))
Method of diagnosing a -786C/T variance in the eNOS

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes III and IV.

gene by means of RFLP or FRET analysis. Kit for implementing the method.

Invention 19 (claim 10 (in part))
DNA oligonucleotide with a nucleic acid sequence according to Seq. Id. No. 35.

Inventions 20-30 (claim 10 (in part))
As Invention 19, but in each case relating to one of Seq. Id. No. 36-40 and 56-61.

1.2 The reasons therefor are as follows:

a) the only technical feature that all inventions have in common is the reference to the human eNOS gene, in particular to its T-786C polymorphism. Inventions 1-17 relate to decoy oligonucleotides that contain said polymorphism and thus represent allele-specific oligonucleotides. Invention 18 relates to the detection of said polymorphism. Inventions 19-30 relate to oligonucleotides that hybridize with the mRNA or the promoter sequence of the human eNOS gene.

b) D1 discloses double-stranded oligonucleotides which in each case represent an allele of the T-786C-eNOS polymorphism, where the oligonucleotide matching the C allele binds RPA1 specifically. It is also shown that the RPA1 expression correlates with transcriptional hypoactivity of the -786C-eNOS allele. The double-stranded oligonucleotides

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes III and IV.

according to D1 are decoy oligonucleotides in the stricter sense. D2 discloses the T-786C-eNOS polymorphism as well as methods of detecting it. Also disclosed are single-stranded oligonucleotides with specificity for the human eNOS gene. These oligonucleotides contain *inter alia* the T-786C polymorphism and are specific to one of its alleles. Since the oligonucleotides according to claim 1 can be either double- or single-stranded, the oligonucleotides according to D2 are, by their allele specificity, decoy oligonucleotides in terms of claim 1. D3 discloses the T-786C-eNOS polymorphism as well as methods of detecting it by means of PCR, using eNOS-specific primers and allele-specific oligonucleotide samples. For the above-mentioned reasons, said allele-specific oligonucleotides are decoy oligonucleotides in terms of claim 1. D4 discloses a method of detecting the T-786C-eNOS polymorphism by means of RFLP. D5 teaches a quantitative method of detection of eNOS-mRNA by means of TaqMan-real time PCR using oligonucleotides that bind to the mRNA of the human eNOS gene.

c) In light of the prior art represented by D1-D5, the technical problem addressed by the present application can be defined as 1) providing further decoy oligonucleotides with specificity for the T-7867C polymorphism (inventions 1-17), 2) providing further methods of detecting the T786C-eNOS polymorphism as well as a kit for implementing said

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes III and IV.

methods and 3) providing further eNOS-specific oligonucleotides.

d) Each of the above-indicated inventions represents an independent solution to one of the problems addressed by the application. Solution 1 provides decoy oligonucleotides according to Seq. Id. No. 1 and 2. Solutions 2-17 provide decoy oligonucleotides characterized by two successive Seq. Id. Numbers from Seq. Id. No. 3-34. Solution 18 provides a method of detecting T-786C-eNOS polymorphism by means of RFLP or FRET analysis of PCR amplificates as well as kit for carrying out these methods. Solutions 19-30 provide in each case an eNOS-specific oligonucleotide according to one of Seq. Id. No. 35-40 as well as 56-61.

e) In view of the fact that the T-786C-eNOS polymorphism, methods of detecting it, oligonucleotides with specificity for the promoter and the mRNA of the human eNOS gene as well as decoy oligonucleotides with specificity for said polymorphism are already known from the prior art, since furthermore the oligonucleotides of solutions 1-17 and 19-30 differ greatly in the part of their primary structure that deviates from the prior art, and since no other shared technical features can be identified which in light of the prior art can be regarded as special technical features common to the above-mentioned solutions, the IPEA is of the view that the thirty solutions contained in the

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes III and IV.

present application are not based on a single general inventive concept within the meaning of PCT Rule 13.1. Hence, unity of invention is lacking and the different inventions are as listed above.

f) The ISA has searched the first invention.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-3	YES
	Claims		NO
Inventive step (IS)	Claims	2, 3	YES
	Claims	1	NO
Industrial applicability (IA)	Claims	1-3	YES
	Claims		NO

2. Citations and explanations

1. The subjects of claims 1-3 are novel because none of the documents of the prior art proposes an oligonucleotide according to Seq. Id. No. 1 or 2.

2.1 D1 discloses a double-stranded oligonucleotide, M1, that matches the -786C allele of the T-786C-eNOS polymorphism (page 2635, left-hand column, paragraph 5, fig. 1c). In EMSA tests this oligonucleotide binds the repressor RPA1. With 31 nucleotides, its length matches the length of decoy oligonucleotides according to the present application. Based on its binding of the repressor RPA1 and from the fact that it contains the polymorphous position of the T-786C-eNOS polymorphism, said M1 oligonucleotide is a decoy oligonucleotide in the sense of the present application. The decoy oligonucleotide according to claim 1 differs from M1 in its sequence. The technical problem therefore consists in providing an alternative RPA1-binding oligonucleotide. The oligonucleotide according to claim 1, however, is only one of many possible alternatives for selection by a person skilled in the art, the selection of which, however, does not involve an inventive step

because it does not entail a surprising effect (PCT Article 33(3)).

D2 (Seq. Id. No. 9-12) and D3 (page 2865, right-hand column, fig. 3) disclose oligonucleotides with specificity for the -786C allele of the T-786C-eNOS polymorphism. Since claim 1 appears to relate to single- and double-stranded oligonucleotides, the above arguments can also be applied on the basis of D2 or D3 to contest inventive step in claim 1 (PCT Article 33(3)).

2.2 The subject matter of claim 2 differs from D1 in the oligonucleotide sequence and the use of the oligonucleotide as drug. The technical problem consists in providing an alternative oligonucleotide for use as a drug. Whereas the oligonucleotide by the above argumentation is not inventive per se, still no prior art document suggests using this oligonucleotide as a drug (PCT Article 33(3)).

2.3 Similar arguments apply to claim 3 (PCT Article 33(3)).

3. The subjects of claims 1-3 appear to have industrial applicability (PCT Article 33(4)).

4. Double-strandedness appears to be an essential feature of the decoy oligonucleotides.

Since claim 1 does not contain this feature, it does not meet the requirement of PCT Article 6 in conjunction with PCT Rule 6.3(b) that every independent claim must contain all technical features Th are necessary for the definition of the invention.